

Eliminating Toxicity of Smoking Through Tea



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Emphysema is a major and increasing global health problem which is at present the fourth and predicted to become the third commonest cause of death and fifth commonest cause of disability in the world by 2020 (source: WHO)

Causes of emphysema:

1. 90% of emphysema is caused by **cigarette smoking**
2. 7% is caused by environmental pollution
3. 3% is due to genetic defect (deficiency of antitrypsin enzyme in the lung)

Global Prevalence of Emphysema *

| Country | Prevalence of emphysema | Population |
|------------|-------------------------|---------------|
| USA | 2,159,230 | 293,655,405 |
| Canada | 239,028 | 32,507,874 |
| Britain | 443,166 | 60,270,708 |
| Australia | 146,420 | 19,913,144 |
| INDIA | 7,831,401 | 1,065,070,607 |
| Bangladesh | 1,039,268 | 141,340,476 |
| China | 9,550,349 | 129,884,7624 |

*About 1 in 136 people (approx $\approx 0.74\%$).

US CENSUS BUREAU, International data base, 2010.

What is emphysema?

Emphysema is defined as a pathological condition of lung characterized by

- Permanent enlargement of airspaces
- Accompanied by destruction of their walls
- Loss of lung elasticity
- Causes impaired transfer of oxygen and carbon dioxide into and out of the blood



Some Facts About Emphysema

- Emphysema cause irreversible damage of the lung
- There are no effective cures for emphysema
- Only palliative treatments are available which reduces the symptoms of the disease.
- So it is important to explore strategies for it prevention

Animal model to understand pathogenesis and mechanism of emphysema

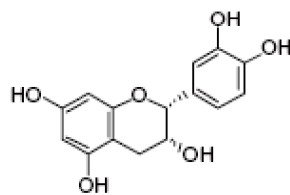
- **90% of emphysema is caused by cigarette smoking.**
So, the best model should be cigarette smoke driven animal model
- Guinea pig is a good animal model. This is because the structure of the guinea pig lung and alterations after CS exposure have similarity with that of human lung
- Until now it takes 8-12 months to produce cigarette smoke-induced emphysema in guinea pigs. But maintaining sub-clinically deficient conditions emphysema can be induced in guinea pigs within a shorter period

Our work reveals that cigarette smoke-induced emphysema is primarily caused by oxidative damage of lung proteins by oxidant(s) present in the cigarette smoke

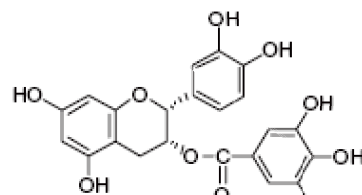
ANTI-OXIDATIVE COMPONENTS OF BLACK TEA

Components of black tea with antioxidant properties

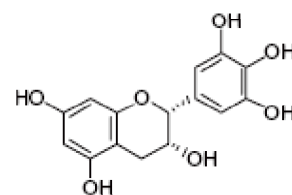
Theaflavins, Epigallocatechin (EGC), Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epicatechin & Catechin



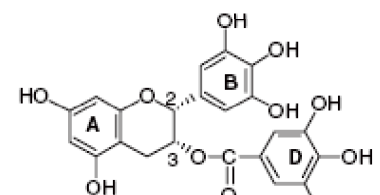
(-) epicatechin (EC)



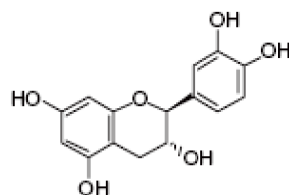
(-) epicatechin gallate (ECG)



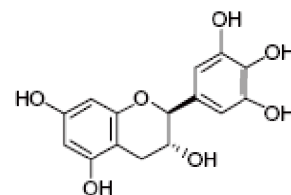
(-) epigallocatechin (EGC)



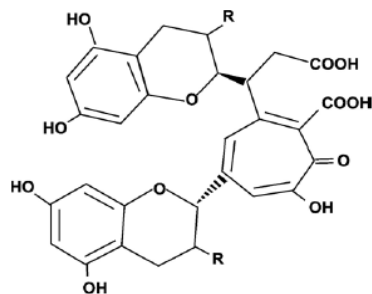
(-) epigallocatechin gallate (EGCG)



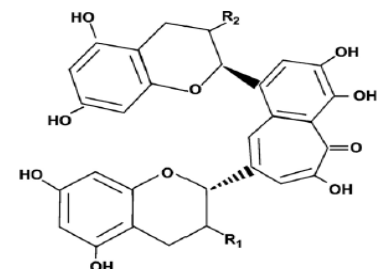
(+) catechin



(+) gallocatechin (GC)



(R=Galloyl or other groups)



Theaflavin [TF₁]: R₁ = R₂ = OH
Theaflavin-3-gallate [TF₂A]: R₁ = Galloyl; R₂ = OH
Theaflavin-3'-gallate [TF₂B]: R₁ = OH; R₂ = Galloyl
Theaflavin-3,3'-digallate [TF₃]: R₁ = R₂ = Galloyl

SCHEME OF ANIMAL TREATMENT

EXPERIMENTS



Guinea pigs
kept on synthetic
diet

EXPOSED TO AIR

**EXPOSED TO CIGARETTE
SMOKE**

**EXPOSED TO CIGARETTE
SMOKE & GIVEN BLACK
TEA**

Dose of CS: Two puff/cigarette
5 cigarettes/guinea pig/day

Lung Protein Oxyblot Showing Prevention of Cigarette Smoke-induced Protein Oxidation by Black Tea

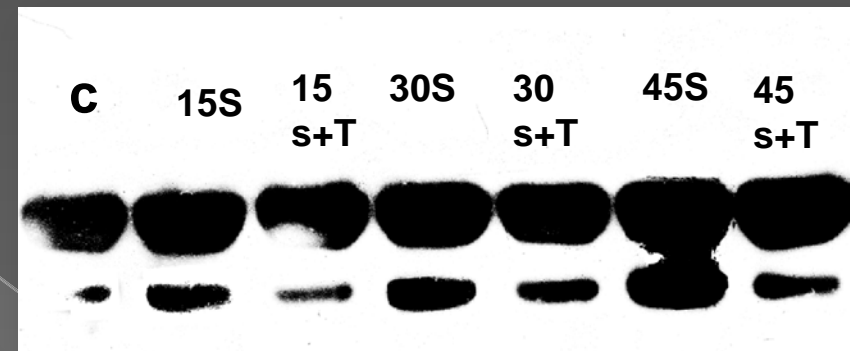
After treatment of the animals for relevant periods with and without Black Tea supplementation (here 45 days), lung proteins were subjected to oxyblot assay against untreated control. The results show prominent oxidative damage due to CS exposure and significant prevention of the same by Black Tea.

Result shown is representative of three independent experiments carried out under identical conditions with 8 animals in each group.



Cigarette Smoke Induced Serum Protein Oxidation and Protection by Black Tea (BT)

After CS treatment of the animals for 15, 30 and 45 days with and without BT supplementation, serum samples were collected and subjected to Oxyblot assay against untreated control. The results show prominent oxidative damage due to CS exposure and protection of the same by BT.

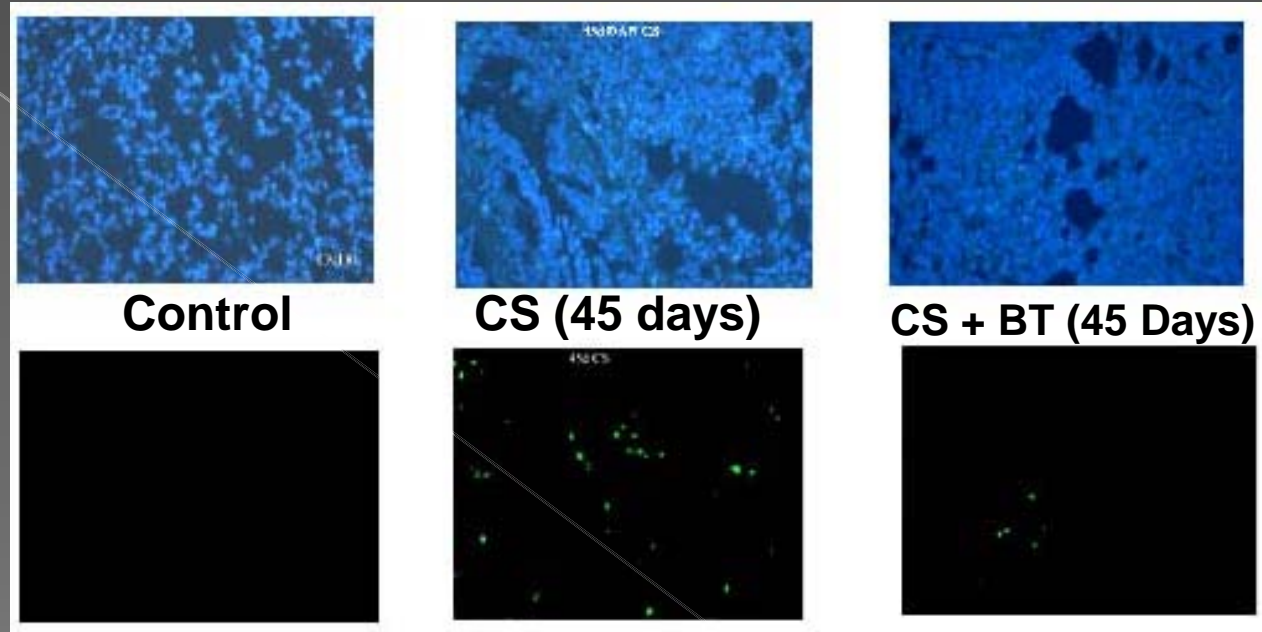


Black Tea Prevents Cigarette Smoke-induced Apoptosis of Lung Cells in treated Guinea Pigs (TUNEL Assay)

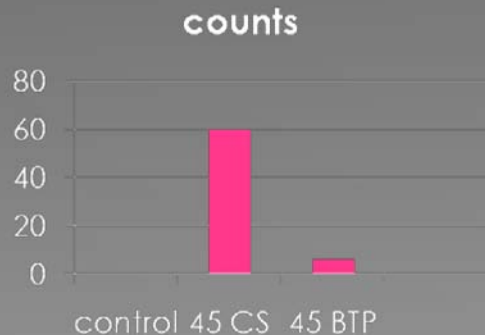
Nuclei counterstained with DAPI

Marked increase in the TUNEL positive cells in the lung tissues of CS exposed guinea pigs given water as the drink (lower panel) was observed, as indicated by green fluorescence attributable to fluorescein-dUTP labeling of damaged DNA.

TUNEL positive cells (apoptosis) decreased significantly with BT given to CS-exposed guinea pigs as the drink

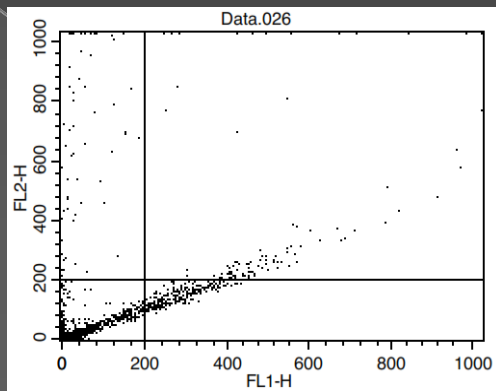


Cells with TUNEL Stain

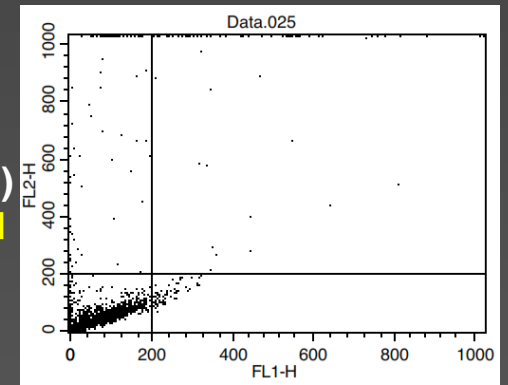


Supplementation of BT prevents CS-induced apoptosis in the guinea pig lung

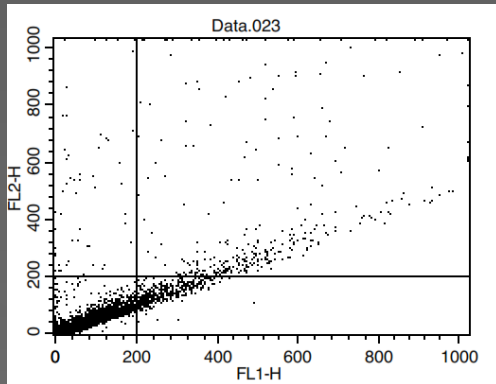
**Fluorescence Activated Cell Sorting (FACS)
Analysis of Annexin V and PI labeled blood
cells collected from experimental animals**



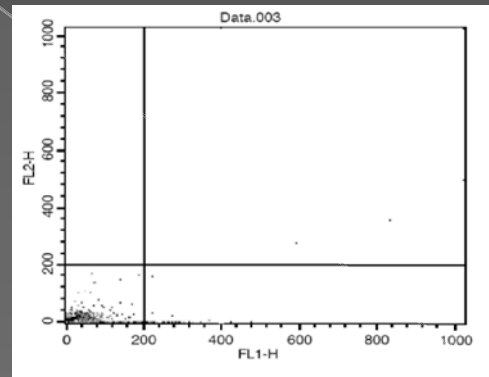
Cigarette Smoke (15 Days)



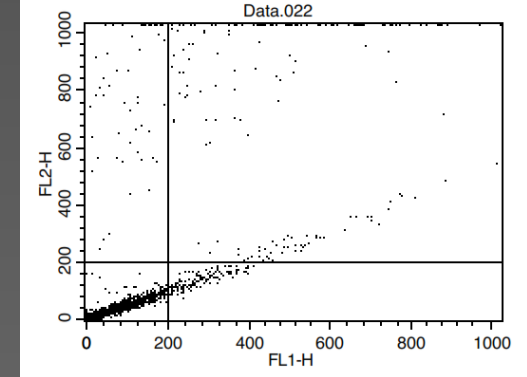
Cigarette Smoke + BT (15 Days)



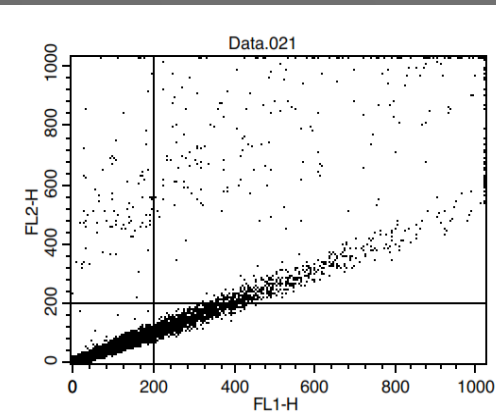
Cigarette Smoke (30 Days)



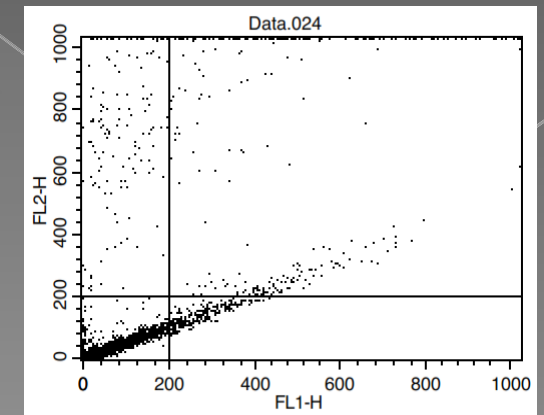
Control



Cigarette Smoke + BT (30 Days)



Cigarette Smoke (45 Days)



Cigarette Smoke + BT (45 Days)

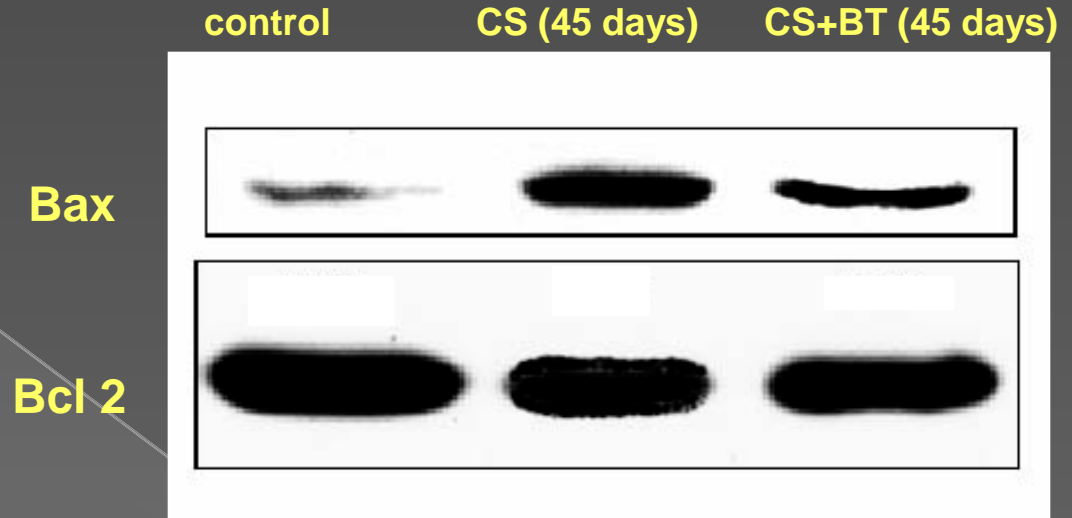
Black Tea Protects against Cigarette Smoke-induced Apoptosis of Lung Cells In Treated Guinea Pigs

(Bax/Bcl2 Analysis)

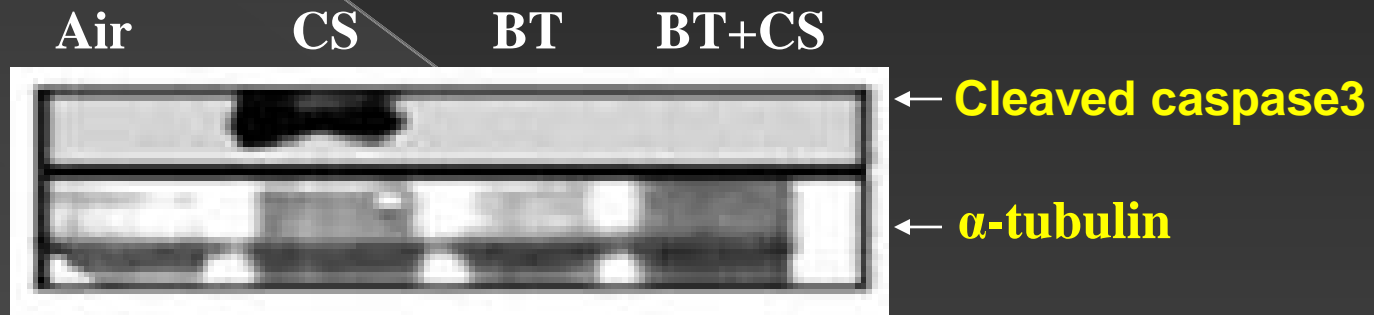
Over-expression of Bax, a member of the Bcl-2 family is an established indicator of cellular apoptosis.

While Bax is proapoptotic, Bcl-2, is antiapoptotic.

While the level of Bax protein significantly increased in response to CS treatment given water as the drink, lesser change of this protein was observed in the animals supplemented with BT during CS exposure.

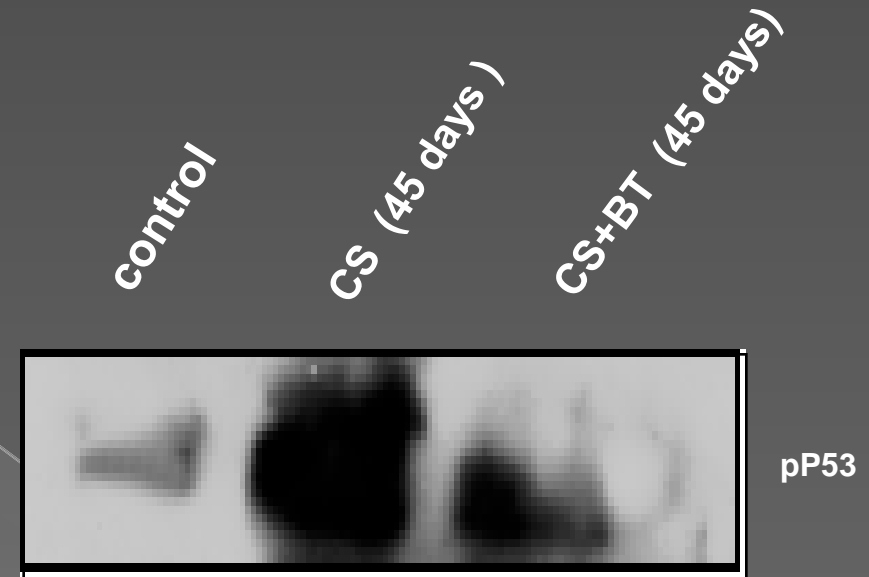


Immunoblot of caspase 3 of the lung extracts of guinea pigs exposed to air or CS and given water or BT as the drink demonstrate that black tea prevents activation of caspase 3



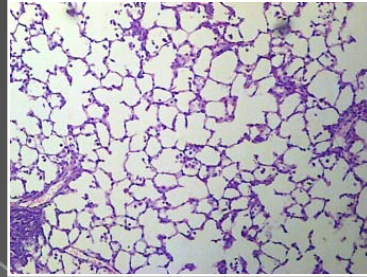
Black Tea Prevents Cigarette Smoke-induced Apoptosis of Lung Cells In Treated Guinea Pigs (p53 Phosphorylation)

Levels of phosphorylated p53 was found to markedly increase in the lungs of guinea pigs exposed to CS (lane 2) and given water as the drink against CS-unexposed animals only given water as drink (lane 1).



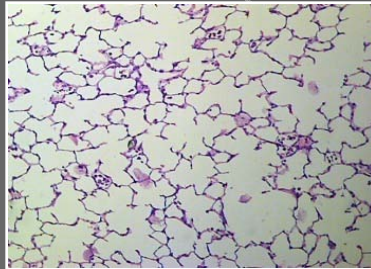
Comparatively lower increase in the phosphorylation levels in lung p53 was observed in CS-exposed guinea pigs given BT as the drink (lane3)

Black Tea Prevents Cigarette Smoke-induced Emphysematous Lung Damage In Treated Guinea Pigs (Histopathological Analysis)

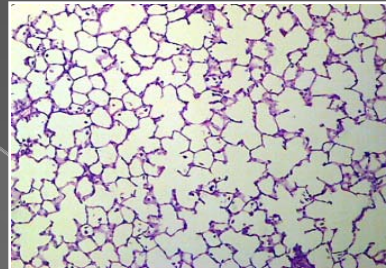


CONTROL

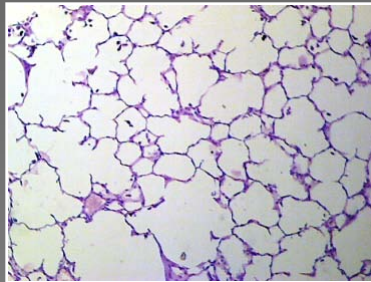
CS-15 DAYS



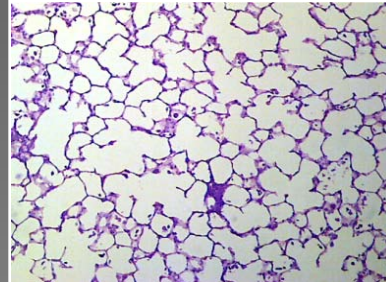
CS+ BT- 15 DAYS



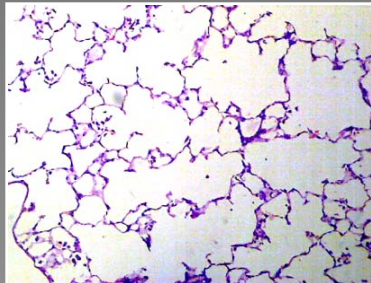
CS- 30 DAYS



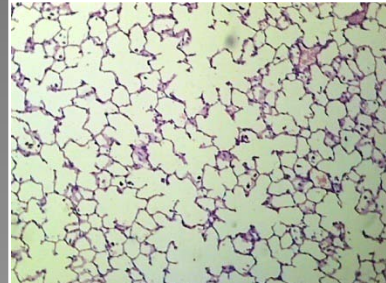
CS+ BT- 30 DAYS



CS- 45 DAYS

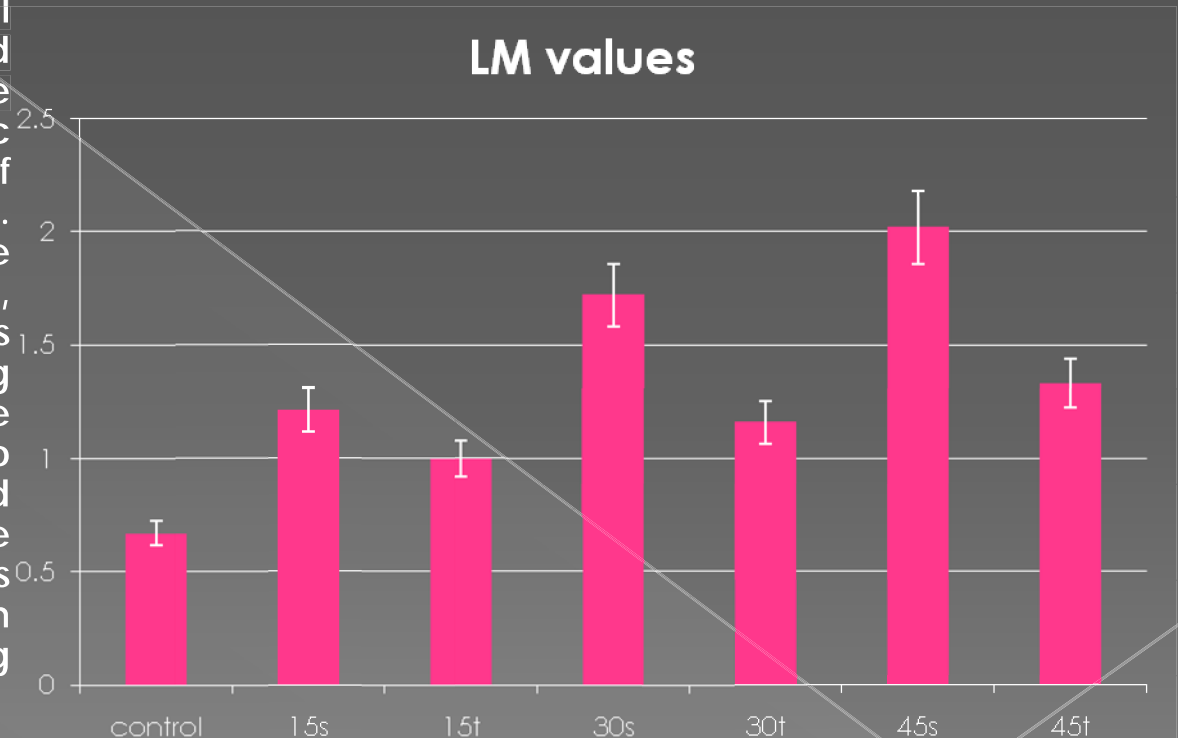


CS+ BT- 45 DAYS



LINEAR MEAN INTERCEPT (LM)

Histology profiles of guinea pigs exposed to CS for 15, 30 and 45 days, showed marked time dependent emphysematous damage of the lung, as compared with sham control guinea pigs exposed to air and given water to drink. The damage was evidenced by morphometric change and enlargement of alveolar air spaces in the lung. Guinea pigs exposed to smoke and given BT as a drink, significantly showed lesser lesions (airspace expansion) in the lung cells and the histological profile appeared to be more similar to that of normal, unexposed guinea pigs. Quantitative evaluation of lung damage was done by measuring the mean Lm of the inflated lung sections using the Dewinter Biowizard software.



SUMMARY

- Cigarette smoke causes extensive oxidative damage of lung proteins causing emphysema
- Black tea antioxidants prevents cigarette smoke induced oxidation of lung proteins and thereby protects against cigarette smoke induced emphysema
- Our work reveals the capability of black tea to combat xenobiotic induced oxidative damage and its potential to act as a potent preventive against the diseases implicated with oxidative damage including senile degeneration.

THANK YOU